REMARKS

Applicants, through the undersigned, wish to thank Examiner Crouch for the courtesy and assistance extended on behalf of Applicants during a personal interview conducted on April 21, 2005.

In the Final Action dated December 16, 2004, claims 65-77 are pending and under consideration. Claims 65 and 67-77 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by or, in the alternative, under §103(a) as allegedly obvious over Carpenter (WO99/11758). Claims 65 and 66 are also rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Carpenter in view of Martinez-Serrano et al (*J. Neurosci.* 15: 5668-5680, 1995).

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Applicants respectfully submit the Declaration of Dr. Alan Colman (**Exhibit 1**) and the Declaration of Dr. Clive Niels Svendsen (**Exhibit 2**). Both declarations support the patentability of the claimed invention and are further discussed hereinbelow.

The Examiner has rejected claims 65 and 67-77 under 35 U.S.C. §102(b) or, alternatively, under §103(a) based on Carpenter (WO99/11758). The Examiner alleges that Carpenter teaches transplantation of human ES-derived neural progenitor cells isolated from embryonic human forebrain into the left striatum of rat brain and the production of a stable graft. The Examiner also alleges that Carpenter teaches treatment of neurodegenerative diseases by administering the human ES-derived neural progenitor cells to a patient to replace deficient neurons. The Examiner states that there is no evidence on record that the neural progenitor cells employed in the presently claimed methods have any distinction from the neural progenitor cells of Carpenter. The Examiner indicates that in the instant case, Applicants bear the burden to

provide arguments or evidence to establish that the source of the cells makes a patentable distinction.

Applicants respectfully submit the following which establishes that the neural progenitor cells of the present application are distinguished from the cells disclosed by Carpenter in the developmental history, the capacity to give rise to different neural cell types, and the expression markers.

Developmental History

The neural progenitor cells of the present application are differentiated in an *artificial environment* from human embryonic stem (hES) cells. In contrast, the neural stem cells of Carpenter are differentiated from cells of an embryo in an entirely *in vivo* environment.

It should be noted in the first instance that hES cells, which are the starting material giving rise to the neural progenitor cells of the present application, are themselves an artificial product of an *in vitro* culture system and are unlike any cell in any *in-vivo* state. As disclosed in the present application, hES cells are derived from cells of a developing embryo or the inner cell mass (ICM). By removing the trophectoderm to access the ICM, the "normal" embryonic development environment is disrupted. That is, the ICM is now out of context with the trophectoderm and the blastocyst environment has been destroyed. The isolated ICM is then plated and at this point, it can no longer be considered as a normal embryo. All media and subsequent supplements used are *in vitro* "approximates" that have been found to be supportive of hES cells and do not represent a normal *in vivo* environment.

Although hES cells exhibit some of the properties of cells from an early embryo, hES cells have properties not shared by cells of intact embryo. A particularly notable example is that no pluripotent cell demonstrates long term self-renewal *in vivo*. hES cells are pluripotent and can indefinitely self-renew. In contrast, pluripotent cells of the early mammalian embryo proliferate only briefly before becoming cells with a more restricted developmental potential. In this regard, Applicants respectfully direct the Examiner's attention to the Colman Declaration, ¶¶

7-8. It is Dr. Colman's opinion that the ability of hES cells to proliferate indefinitely in an undifferentiated state *in vitro* is consistent with the notion that hES cells are a tissue culture artifact.

Therefore, it is believed that hES cells are not a natural phenomenon and lack an *in vivo* equivalent. In this regard, Dr. Colman refers to a publication co-authored by J. Thomson, the first person to have developed hES cells. See the Colman Declaration, ¶ 9. The publication questions the notion that hES cells are the equivalent of cells of an early (5 day) embryo.

In this regard, Applicants observe that the Examiner characterizes Carpenter's cells as "human ES derived neural progenitor cells". See page 2 of the Final Action. The Examiner also states on page 5 of the Action that the cells of Carpenter ultimately were derived from human ES cells, as "all human cells are so derived". Applicants respectfully submit that the Examiner's characterizations are inaccurate, as Carpenter's neural progenitor cells are purely developed from cells of early embryos *in vivo*, which are not equivalent to hES cells as utilized in the present application. As submitted, hES cells are not naturally occurring and are considered to be a tissue culture artifact.

Applicants further submit that it is hES cells, which are a tissue culture artifact, that have been utilized in the '543 application to differentiate in an *artificial* environment *in vitro* to obtain neural progenitor cells. In contrast, the neural stem cells described in Carpenter are isolated from the forebrain of a developing embryo. That is, the neural stem cells of Carpenter are derived from pluripotent cells of early embryo in an entirely *in vivo* environment with appropriate developmental cues, whereas hES cells are cultured in an artificial *in vitro* environment in the '543 application, which is devoid of spatial and physiological cues. In this regard, Applicants respectfully direct the Examiner's attention to the Colman Declaration, ¶12.

Thus, the hES-derived NPCs of the present application and the NSCs isolated from the forebrain in Carpenter, have substantially different developmental history, both in terms of the starting material, and the environmental conditions under which the starting cells develop. Applicants further submit that, as a direct or indirect consequence of the different development

histories, the NPCs of the present application and the NSCs of Carpenter also differ in their capacity to give rise to different neural cell types.

Capacity to give rise to different neuronal cell types

Applicants respectfully submit that the neural progenitor cells of the present application are derived from hES cells, an artificial source, in an entirely artificial environment, without any influence of an *in vivo* environment with appropriate developmental cues. In contrast, the NSCs of Carpenter, obtained from an *in vivo* environment (i.e., forebrain), are already regionally imprinted and are both biased and pre-disposed to differentiate into a neuronal cell type of that region (i.e., forebrain).

Applicants respectfully direct the Examiner's attention to the Colman Declaration, ¶¶10-12. As explained by Dr. Colman, when neurospheres derived from different regions of the brain are differentiated *in vitro,* or after transplantation *in vivo*, the cell types that arise have properties and identities which reflect the region of the brain from which they were originally taken – even after extended passaging. Thus, neurospheres generated from the striatum, mesencephalon or cortical regions of the embryonic fetal brain give rise to different cell populations when plated under identical conditions *in vitro*, possessing subtly different properties according to the time and site of origin in the brain.

The differences between the NPCs that are derived from hES cells *in vitro* and the NSCs that are obtained from human fetal tissue, especially in the ability to give rise to different neural cell types, are further substantiated by the Svendsen Declaration. As stated in the Svendsen Declaration, ¶ 7, human neural progenitor cells derived from human fetal cortex (i.e., part of forebrain) are regionally specified at the time of dissection from the developing cortex to make cortical neurons. There are no reports of generating large projection neurons associated with the midbrain or spinal cord (i.e. dopamine neurons, motor neurons, serotonin neurons) from these neural progenitor cells. According to Dr. Svendsen, this is because the progenitor cells

isolated from the cortex at that stage of development have already been specified to produce cortical tissues. See the Svendsen Declaration, ¶ 12.

On the other hand, hNPCs derived from hES cells have enormous plasticity with regard to the types of neuron they can produce. Dopamine neurons and motor neurons with all of the correct physiological characteristics can be generated from hES-derived NPCs. See the Svendsen Declaration, ¶ 15. Therefore, it is Dr. Svendsen's opinion that hES-derived neural progenitor cells and neural progenitor cells derived from fetal tissue are in no way comparable and are distinctly different cells. See the Svendsen Declaration, ¶ 16.

In this connection, Applicants respectfully submit that the NPCs of the present application express tyrosine hydroxylase (characteristic of dopaminergic neurons) after differentiation under appropriate, fairly simple culture conditions. See, e.g., page 82, line 2 of the specification and Figure 28. In contrast, there is an absence of tyrosine hydroxylase+ (TH+) neurons after differentiation of the "Carpenter" cells, unless cytokines (IL1b) are added. See page 271, paragraph 3, of Carpenter et al., *Exp Neurology* 158: 265-278 (1999)[1], attached to the Colman Declaration as Exhibit G. See also the Colman Declaration, ¶13. The Carpenter cells (following differentiation) appear to be enriched for GABA-ergic neurons. GABA is a non-specific forebrain marker.

Expression Markers

Applicants further respectfully submit that hES derived NPCs of the present application and the NSCs derived from fetal forebrain described in Carpenter, are also distinguished in their expression markers. Most notably, the Carpenter cells are positive for low levels of GFAP and beta 3 tubulin, as indicated in Carpenter's application (WO 99/11758) on page 3, line 14. In contrast, as discussed in the Colman Declaration, ¶ 14, the NPCs of the present application are negative for GFAP expression.

[1] This article, authored by Carpenter's group and published after the filing date of Carpenter's International Application (WO 99/11758), also describes the experiments disclosed in WO 99/11758.

Based on the foregoing, Applicants respectfully submit that the neural progenitor cells of the present application are distinguished from the cells disclosed by Carpenter.

Applicants respectfully submit that the source of the cells, i.e., hES-derived NPCs, further define the presently claimed methods of transplantation as a patentable invention. In particular, Applicants respectfully submit that the determination of the present inventors is surprising, namely, the artificially produced hES-derived NPCs, when introduced into an *in vivo* environment, form stable grafts and give rise to three fundamental neural lineages. See, e.g., pages 93-95 of the present specification. Unlike cells derived from much more mature neural tissue, such as the forebrain tissue utilized by Carpenter, that had been subjected to the appropriate neural environmental cues during neural development, the hES derived NPCs have had no spatial relationships with surrounding tissues. Applicants also direct the Examiner's attention to the Colman Declaration, ¶13, in this regard.

Applicants further respectfully submit that there is no suggestion in Carpenter to utilize hES-derived NPCs in transplantation. In addition, those skilled in the art would not have had any reasonable expectation of success in utilizing hES-derived NPCs in transplantation, as these artificially produced cells would not have had the benefit of any *in vivo* environmental cues. In contrast, it is not surprising that the cells of the Carpenter reference are able to survive in an *in vivo* neural environment, because this is where they essentially come from.

In view of the foregoing, Applicants respectfully submit that the claimed methods are not anticipated by or rendered obvious by Carpenter. Withdrawal of the rejection based on Carpenter is respectfully requested.

Claims 65 and 66 are also rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Carpenter in view of Martinez-Serrano (*J. Neurosci.* 15: 5668-5680, 1995).

The Examiner concedes that Carpenter does not teach injection of neural progenitor cells into a lateral cerebral ventricle. However, the Examiner contends that Martinez-Serrano teaches the implantation of CNS derived neural progenitor cells into the septum of a rat brain to prevent significant loss of cholenergic cells.

Applicants respectfully submit that, as established hereinabove, the hES-derived neural progenitor cells of the present application are distinguished from the neural stem cells isolated from fetal tissue, disclosed by Carpenter. Similarly, the hES-derived neural progenitor cells of the present application are distinguished from the neural stem cells isolated from adult neural tissues, such as those disclosed by Martinez-Serrano.

Applicants further respectfully submit that because of the artificial *in-vitro* nature of human ES cells and the NPCs derived therefrom, a surprising aspect of the presently claimed methods is the fact that transplantation of such NPCs succeeded at all. The hES-derived NPCs have had no spatial relationships with surrounding tissues as have the fore-brain derived NSCs of Carpenter, or the CNS derived NSCs cells of Martinez-Serrano, or the NPCs from human embryonic brain tissue of Fricker (*J. Neurosci.* 19, 5990-5005, 1999, cited in the previous Action). The hES derived NPCs have had a unique and artificial developmental history, and it is surprising that when introduced back into an *in-vivo* environment, they form stable grafts at all and give rise to several neural cell types.

Accordingly, Applicants respectfully submit that the claimed methods are not obvious over Carpenter and Martinez-Serrano. Withdrawal of the rejection based on Carpenter and Martinez-Serrano is respectfully requested.

Applicants further respectfully submit that in an effort to advance prosecution, independent claim 65 has been amended to recite "central nervous system", in place of "nervous system". Support for this amendment is found in the specification, e.g., page 53, line 20. As presently claimed, the claims are directed to methods of transplantation into a host having a central nervous system, which involve obtaining hES-derived NPCs and injecting the NPCs into the central nervous system of the host.

Moreover, claim 72 has been amended to recite "a neurodegenerative disorder" as the pathological condition to be treated. Support for such amendment is found in claim 73. Claim 73 is therefore canceled without prejudice. Applicants further respectfully submit that it is generally known that tyrosine hydroxylase positive (or "TH(+)") cells die off in

neurodegenerative disorders, e.g., Parkinson's. The present application has demonstrated that transplanted NPCs can produce tyrosine hydroxylase (TH+) neurons, which provides support for the claimed method for treating a neurodegenerative disease. Additional support for the claimed method is also found in an article by Ben-Hur et al. (**Exhibit I** attached to the Colman Declaration), reporting that transplantation of hES-derived NPCs improved behavioral deficit in Parkinsonian rats.

It is respectfully submitted that the foregoing amendments to the claims are fully supported by the specification. No new matter is introduced. Applicants reserve the right to pursue the subject matter embodied in the claims as originally filed in a continuation application.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

Xiaochun Zhu
Registration No. 56,311

Scully, Scott, Murphy & Presser
400 Garden City Plaza-Ste 300
Garden City, NY 11530
Telephone: (516) 742-4343
XZ:ab

Encls.:
Colman Declaration (Exhibit 1, with Exhibits A-K attached thereto);
Svendsen Declaration (Exhibit 2, with Exhibits A-F attached thereto).